Highly specific target recognition by an Argonaute protein from Rhodobacter sphaeroides

P.01-076-Mon

D. Esyunina[I], M. Ninova[II], A. Aravin[II], A. Kulbachinskiy[I]

[I]Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia, [II]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, United States of America

Small noncoding RNAs play essential roles in genetic regulation in eukaryotic cells. In particular, several classes of small RNAs associate with proteins from the Argonaute (Ago) family to suppress the expression of complementary mRNA targets. Many bacteria also have Ago proteins, whose functions and the mechanism of action are poorly understood. Homology of prokaryotic Ago's with eukaryotic proteins makes them a promising model for structural and functional studies of the interference mechanisms. In our work we focused on the Ago protein from *Rhodobacter sphaeroides* (RsAgo) that was recently shown to use small RNA guides for targeting DNA. We demonstrated that RsAgo binds guide RNA (gRNA) with high affinity and found a single amino acid residue that participates in the specific recognition of the guide 5'-nucleotide (U). We also measured the affinity of the RsAgo-gRNA complex to single-stranded target DNAs (tDNAs) with either full complementarity to the guide or with mismatches at each position. We found that fully complementary tDNA is bound with very high affinity, while mismatches both in the seed region close to the guide 5' end and in the downstream guide part significantly decrease target binding. Complexes of RsAgo with only gRNA or with gRNA and complementary tDNA are stable and reveal very limited exchange of nucleic acids over time. At the same time, we found that mismatches between gRNA and tDNA lead to dissociation of the guide-target duplex from the RsAgo protein. We propose that this may serve as a mechanism for nucleic acid exchange in the cell, promoted by Ago interactions with non cognate targets. Our results reveal how complementarity between the guide and target strands affect the efficiency of target recognition and suggest a potential mechanism for guide release and Ago recycling. This work was supported in part by the Russian Science Foundation (grant 16-14-10377).